Cold disinfestation of capsicum and chilli fruit from Queensland fruit fly

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NSW Department of Primary Industries

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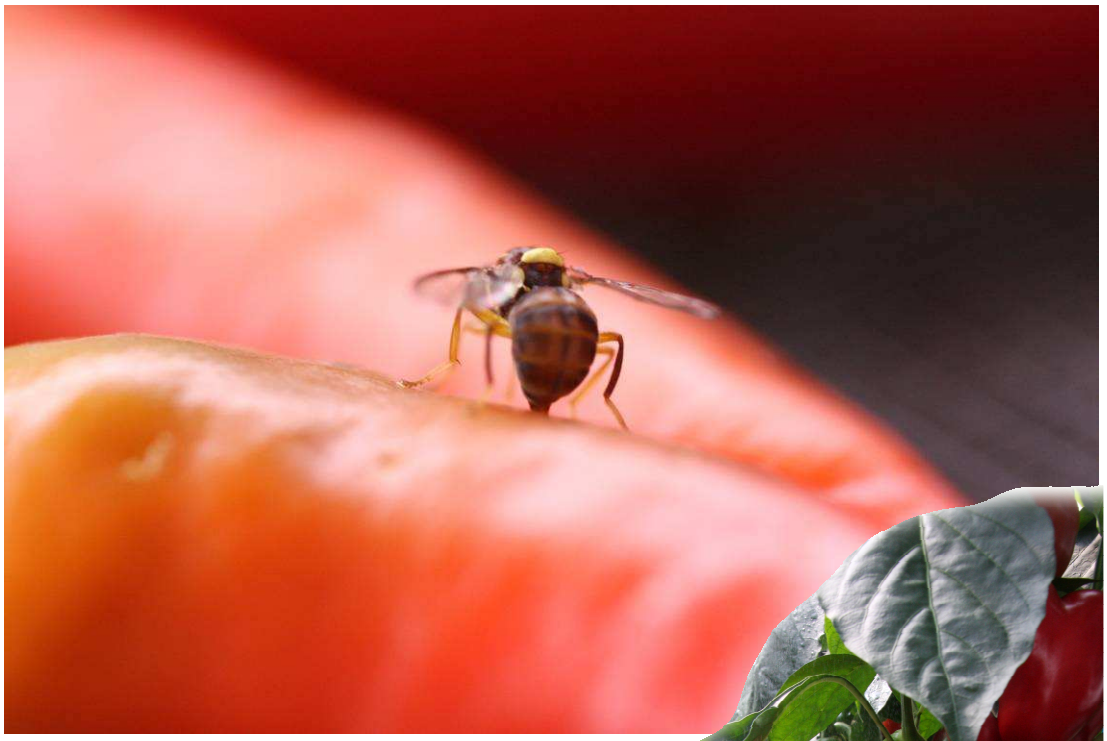

Final Report

Project VG10028

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Penta Pristijono

NSW GOVERNMENT Department of Primary Industries

AUSVEG

HAL Horticulture Australia

VG10028 - Cold disinfestation of capsicum and chilli fruit from Queensland fruit fly

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Purpose of this report :

Capsicums and chillies grown in areas with Queensland fruit fly (Qfly) but destined for markets free of this pest must meet quarantine protocols. Many growers previously met Interstate and International requirements by flood-spraying postharvest with either dimethoate or fenthion insecticide. Both treatments have now been withdrawn for use on capsicums, with only fenthion currently permitted for chillies.

This project has developed a cold disinfestation treatment for capsicums and chillies. To reduce sensitivity to cold, the fruit was showered with 55^{o}C water for 60 seconds before cold treatment.

Mortality data during 3^{o}C storage, with and without the hot water shower treatment, is presented for all Qfly life-stages (egg, 1st instar, 2nd instar and 3rd instar larvae) in 2 varieties of chillies as well as capsicums. The results demonstrate that the short heat treatment increased, rather than decreased the efficacy of the cold treatment. Data is further presented from four large scale trials on capsicums and cayenne chillies. All experiments were replicated in time. The estimated number of treated insects with no survivors exceeds that required by the Japan Ministry of Agriculture, Fisheries and forestry, that being a generally accepted high international standard. This data may be used to establish a new ICA for interstate access as well as to support international market access applications.

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Contents

Media Summary

Queensland fruit fly (Qfly) is often called Australia's worst horticultural pest. Capsicums and chillies are particularly suitable hosts, so fruit grown in endemic areas must be treated to ensure they are Qfly free before sale in areas free of this pest - such as Victoria, SA, WA and export markets.

Until recently the most common method of ensuring fruit were fruit fly free was postharvest treatment with dimethoate insecticide. However, this product has now been withdrawn from this use and growers must find an alternative method.

Storage at 3^{o}C or lower is widely used as a quarantine treatment against fruit flies for other fruit and vegetables. However, the method has never been tested for capsicums and chillies as they are tropical fruit, sensitive to the low temperatures required. Recent research has demonstrated that a hot water shower (HWS) can reduce cold sensitivity, allowing capsicums and chillies to be stored at suitably low temperatures for several weeks.

The aim of this project was to develop a quarantine treatment against Qfly for capsicums and chillies, with and without the addition of a HWS (60 seconds at 55^{o}C).

Initial tests showed that there was no difference between green and red capsicums in either attractiveness to female Qfly or suitability for larvae development. Red and green capsicums, cayenne chillies and birdseye chillies could therefore be used to test which Qfly life stage (egg, 1st, 2nd or 3rd instar larvae) survived the longest during storage at 3^{o}C. We then repeated the same tests but with the addition of a HWS.

First instar larvae could survive longer at 3^{o}C than any other lifestage, regardless of whether they were treated with a HWS. The HWS slightly reduced the storage time required to kill the larvae, especially for the older lifestages tested. Promisingly, around 50% of all larvae were killed using a HWS alone. No larvae survived when chillies and capsicums were stored for more than 7 days at 3^{o}C.

The treatment was then tested by storing larger volumes of infested capsicums and cayenne chillies at 3^{o}C for 10 days. Eight trials were conducted, including two where fruit was HWS treated before storage. From survival rates in untreated fruit, it was estimated that at least 171,511 1st instar larvae were treated. No survivors were found from any of the trials. This demonstrated that 10days at 3^{o}C provides an extremely high level of quarantine security against Qfly.

Finally, HWS effects on fruit quality were tested in a commercial setting. Trials were conducted at AustChilli in Bundaberg with 6 chilli and 2 capsicum varieties. Fruit were stored for either 6 or 16 days. The HWS reduced rots and increased the number of marketable fruit, but did not fully compensate for the longer storage time. The exercise illustrated some practical difficulties with application of the method. The HWS is most likely to be useful where products are especially chilling sensitive or very long storage times are required.

Technical Summary

Queensland fruit fly (*Bactrocera tryoni* Froggatt) (Qfly) is generally regarded as Australia's worst horticultural pest. Capsicums and chillies (*Capsicum annuum* var.) are potential hosts, so fruit grown in endemic areas must be subjected to a quarantine treatment to access markets free of this pest including Victoria, SA, WA and export markets.

Until recently postharvest insecticide flood spraying was the most common method used to meet interstate and international requirements. However, registration of dimethoate for this use pattern has now been withdrawn, as has postharvest application of fenthion to capsicums.

Cold storage at 3^{o}C or lower is widely used as a quarantine treatment for other fruit and vegetables. The method has never been tested for capsicums and chillies as they are considered chilling sensitive and not recommended to be stored below 7^{o}C. However, recent research has demonstrated that a short hot water shower (HWS) can reduce chilling sensitivity and extend storage life, protecting capsicums and chillies from the negative impacts of a disinfestation protocol.

The aim of this project was to develop a data package suitable to support a quarantine treatment against Qfly for capsicums and chillies, with or without the addition of a HWS (60 seconds at 55^{o}C).

Initial tests showed that there was no difference in either Qfly oviposition preferences or larval survival to pupation between green and red capsicums. Both red and green capsicums, cayenne chillies and birdseye chillies were therefore used to test which Qfly life stage (egg, 1st, 2nd or 3rd instar larvae) was the most cold tolerant at 3^{o}C, with and without the addition of a HWS.

First instar larvae were consistently the most cold tolerant in all three *C. annuum* cultivars tested, irrespective of HWS treatment. It had been thought that the HWS might increase cold tolerance of larvae, but Probit analysis indicated that the HWS actually slightly reduced the storage time required to approach 100% mortality. The HWS alone resulted in 39-79% mean mortality and it is estimated that at least 50% of a natural population would be killed by a HWS treatment. No larvae survived when chillies and capsicums were stored for more than 7 days at 3^{o}C.

Eight large scale trials were conducted, storing infested capsicums and cayenne chillies for 10 days at 3^{o}C. This included two where fruit was HWS treated before storage. It was estimated from untreated controls that at least 171,511 1st instar larvae were treated (at the 95% lower confidence interval). No survivors were found from any of the trials. This demonstrates that 10 days at 3^{o}C exceeds Probit 9 quarantine security against Qfly in *C. annuum* cultivars.

The final trial tested HWS application in a commercial environment. Trials at AustChilli examined quality and shelf life of 6 chilli and 2 capsicum varieties following simulation of normal marketing ± a disinfestation treatment. The HWS significantly reduced rots and increased the number of marketable fruit, but did not fully compensate for an additional 10 days storage. The exercise also illustrated some practical difficulties with application of the method. The HWS method is most likely to be cost effective with more chilling sensitive products than used in this trial or longer, colder storage.

1. Introduction

1.1 Capsicums and Chillies

Capsicums (*Capsicum annuum* L.*)* are a major crop in Australia. Over 60,000 tonnes are produced annually with a fresh market value >$230 million (Freshlogic, 2012). While greenhouse production is increasing, the majority of capsicums (>80%) are still field grown. More than half of Australia's production (~58%) comes from Queensland, with large plantings around Bowen and Bundaberg and smaller summer plantings in the Lockyer Valley and the Granite Belt.

Much of the Queensland production is shipped south, to markets in Sydney, Melbourne and further afield. There are also export markets; until recently New Zealand was an attractive market for field grown capsicums, with Dubai another export destination. HAL project VG01090 (Export evaluation study for the Australian vegetable industry) identified potential new markets for Australian capsicums in Singapore, Malaysia, Hong Kong and even Japan, with fruit fly being the major impediment to accessing these markets.

Chillies (*C. annuum* L.*)* are primarily grown for the domestic market, with production centred on Bundaberg in Queensland. Varieties range greatly in size and taste with 'red cayenne' and the smaller, hotter 'birds-eye' the most popular types grown. Like capsicums, much of the market is in southern states.

1.2 Queensland fruit fly

Both capsicums and chillies are vulnerable to infestation by Queensland fruit fly (*Bactrocera tryoni* Froggatt) (Qfly). The pest is endemic in Queensland and most production areas (Dominiak and Daniels, 2011). While avoiding infestation in the field represents a challenge in terms of maintaining fruit quality, Qfly is also a quarantine concern for markets in Victoria, parts of NSW, Tasmania, South Australia, Western Australia and most export markets.

As a result, capsicums and chillies must be treated to eliminate potential infestation before transport to fruit fly free areas. In the past, susceptible produce were usually flood sprayed with dimethoate insecticide to access both interstate markets and the international New Zealand market. However, in August 2008 the Australian Pesticide and Veterinary Medicine Association (APVMA) suspended postharvest use of this chemical on edible skinned crops including capsicums and chillies.

Until October 2012 packers were able to substitute another insecticide, fenthion, to continue to meet quarantine protocols. However, this chemical has now also been withdrawn from postharvest application on capsicums. Although use is still permitted on chillies, the limited number of horticultural products which can still use this chemical places its long term availability in doubt. Alternative quarantine methods, preferably non-chemical, are therefore required.

1.3 Disinfestation

Cold storage has been commonly used as a treatment against Qfly for many crops, including blueberries, summerfruit, citrus, cherries, avocadoes and grapes (Hill *et al*., 1988, Jessup *et al*., 1993, 1998, Jessup, 1994, Heather *et al*., 1996). Storage at 1-3^{o}C for 14 days or more is accepted as a quarantine treatment for Australian citrus,

summerfruit, table grapes and other crops exported to important markets such as Japan, Taiwan and the USA.

Cold treatments have not been previously developed for capsicums and chillies because these crops originated in the tropics of the Americas and are chilling sensitive. They are not recommended to be stored below 7^{o}C and in practice are stored close to 12^{o}C (Mitchell, 1992). Exposure to temperatures below 3^{o}C for more than a few days can result in weight loss, calyx darkening and decay (Lim *et al*., 2007).

It may be possible to protect capsicums from chilling injury using a short hot water treatment. Such treatments can promote the formation of heat shock proteins, which maintain membrane integrity at low temperatures (Florissen *et al*., 1996) as well as controlling storage pathogens such as *Botrytis cinerea* and *Alternaria alternata* (Fallik *et al*., 1996, Bar-Yosef *et al*., 1999). Gonzalez-Aguilar *et al.* (2000) claimed that a pre-storage 4 minute dip in 53^{o}C water reduced decay and chilling injury of capsicums following storage for up to 4 weeks at 8^{o}C.

Fallik *et al*., (1999) proposed brushing capsicums with 55^{o}C water for 15 seconds to clean and prevent decay during transport and subsequent retail (2 weeks at 7^{o}C + 3 days at 20^{o}C). This method was patented and is now commercially applied in Israel. In Australia, Ekman and Pristijono, (2010) demonstrated excellent protection against chilling injury for both capsicums and chillies using a 30-60 second shower under 55^{o}C water. This treatment could potentially be applied on packing lines using the drenching systems previously used for dimethoate.

These published results suggest that combining a short hot water shower (HWS) with cold storage could provide a viable disinfestation treatment for capsicums and chillies without compromising quality. However, the effects of such a treatment on Qfly cold tolerance was not known.

While heat treatments are used as a quarantine treatment against Qfly in tropical fruit such as lychees, and mangoes (Jacobi *et al*., 1993, Heather et al., 1997), the treatment proposed here is too short to result in full mortality. Thermal conditioning at sub-lethal temperatures has been shown to increase heat tolerance of Qfly eggs, extending the time at 46^{o}C needed to cause 99% mortality (Waddell *et al*., 2000). It therefore seemed possible that, just as a short heat treatment can protect capsicums from cold, Qfly larvae may also gain a similar protection, effectively reducing mortality.

The trials described in this report therefore report on the tolerance of different Qfly lifestages (egg, 1st instar, 2nd instar and 3rd instar larvae) to both cold storage alone and a hot water shower plus cold storage. Trials initially used two chilli varieties which varied widely in size and flesh thickness (birdseye and cayenne) as well as greenhouse grown capsicums (Section 2).

Once the most treatment tolerant lifestage and number of days required to achieve 100% mortality had been determined, large scale disinfestation trials were conducted in both capsicums and cayenne chillies. These aimed to find no survivors among a minimum of 10,000 of the most treatment tolerant lifestage, replicated at least 3 times for each product type (Section 3).

One of the questions raised during project development was whether capsicum maturity, in terms of red colour development, would affect larval development and survival. A series of trials were conducted examining this issue. These are detailed in Appendix 1.

2. Effect of capsicum fruit ripeness stage on Qfly infestability

2.1 Introduction

One concern raised in relation to the trials was whether susceptibility to infestation varied between red and green capsicums. Qfly are known to be strongly attracted to the colours green and yellow (Hill and Hooper, 1984), suggesting they may prefer to oviposit in green capsicums. However, red capsicums have some of the characteristics of ripe fruits, being slightly higher in sugar. They may also be emitting more aromatic volatiles, believed to stimulate oviposition (Dominiak, 2006). In addition, while the physical structure of capsicums (flesh thickness and firmness) changes little during colour development, it seems possible that changes in their internal chemistry could alter their suitability for Qfly development and pupation (Clarke *et al*., 2011).

It was therefore decided to conduct a series of host preference tests comparing the numbers of pupae recovered from red and green capsicums exposed to a standard pest pressure. Host status determination studies have often followed methods proposed by Cowley et al. (1992). One of the proposed tests utilised laboratory cage trials where punctured / non-punctured fruit were exposed to a potential oviposition load of 1 egg/gram of fruit over a 24 hour period. This method has been included in the Asia and Pacific Plant Protection Commission guidelines for testing non-host status of fruit and vegetables to Tephritid fruit flies (APPC RSPM No. 4, FAO 2005) and adopted by the New Zealand Ministry of Agriculture and Forestry (Villagran *et al*., 2012).

This method was further refined by Lloyd *et al*., (in press), who proposed the use of a Host Susceptibility Index to quantify differences in susceptibility and suitability for Qfly infestation. They found significant differences in the susceptibility to Qfly infestation among different varieties of citrus. Jessup *et al* (1998) used a similar method to demonstrate differences between grape varieties.

The aim of this trial was therefore to determine whether green and red capsicums differed in either their attractiveness to female Qflies or their suitability for larval development.

2.2 Method

2.2.3 Fecundity test

All trials used 2-3 week old flies which had been supplied with protein and given ample opportunity to mate. A number of flies were captured and placed briefly in a cool room (5^{o}C) to make them easier to handle. Thirty gravid female flies were randomly selected and ten were placed in each of three ventilated cages (volume>50L) at 26^{o}C with a supply of sugar and water.

An egg cup (perforated plastic container with a small volume of water and a piece of fruit) was placed in each cage to allow the flies to lay eggs. After 24 hours the cups were removed and the eggs carefully counted into petri dishes lined with moist black filter paper. After a further 24 hours at 26^{o}C the eggs were re-counted to determine

hatch rate. This was used to estimate the viable eggs / fly. The whole trial was repeated on 7 separate occasions so as to test flies of different ages and cohorts.

2.2.4 Preference testing

A "choice" test was used, whereby flies had access to one red and one green capsicum. Pesticide free, 'block' type capsicums were grown hydroponically in a NSW DPI greenhouse. At each testing time (n=5), six red and six green fruit were selected which were in good condition and free from any visible bruising, cracks or splits. Each fruit was weighed and "paired" with one of the opposite colour, so as to present similar sized fruit for testing.

The fruit were then placed inside large, ventilated plastic tubs with a supply of food and water (Figure 2.1). Capsicums were either placed with calyx down (n=2) or horizontally (n=3) inside each tub.

Flies were cooled to allow handling as previously. Sufficient mature female flies were counted into each cage to potentially lay up to 1 egg / gram of fruit. The cages were stored at 26^{o}C for 24 hours to allow oviposition. The capsicums were then removed and placed individually inside small ventilated plastic tubs on a bed of vermiculite at 26^{o}C to allow larvae to develop and pupate. Pupae were counted after 10 and 17 days to determine the number of larvae per fruit.

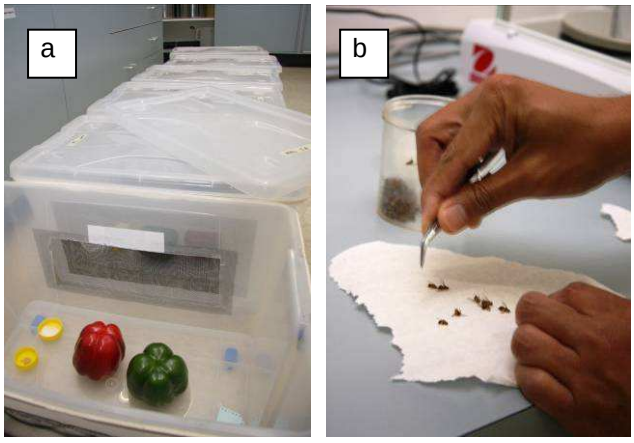

Figure 2.1 - One red and one green capsicum was placed in each ventilated tub with a supply of food and water (a). Sufficient gravid female flies were then released in each tub so as to potentially lay 1 egg/g fruit (b)

2.2.5 Statistical analysis

Data was analysed with CoStat statistical software. An Analysis of Variance using a randomised blocks design was used to test for effect of fruit colour on pupal numbers. Mean values were compared using the Student-Newman-Keuls test at the 95% confidence level.

2.3 Results and Discussion

2.3.1 Fly fecundity

The percentage of eggs which proved viable ranged from 81 to 97%, with an average of 91% hatching within the experimental period.

Each fly laid an average of 55 (std error = 7) viable eggs over the 24 hours of the test. A trend was noted of older flies (~24 days) laying fewer eggs than younger flies (~17

days). However, the data on this was insufficient for proper analysis in the current trial. This could potentially have implications for pest control strategies, so may be worthwhile to test separately.

2.3.1 Differences between red and green capsicums

There were no significant differences in the number of pupae recovered from red and green capsicums (p=0.95), regardless of their position inside the container. However, it was noted that the flies successfully laid more eggs in the capsicums placed on their sides compared to those placed calyx down (Figure 2.2). It was observed that many of the oviposition points were in the soft calyx tissue itself, rather than the skin.

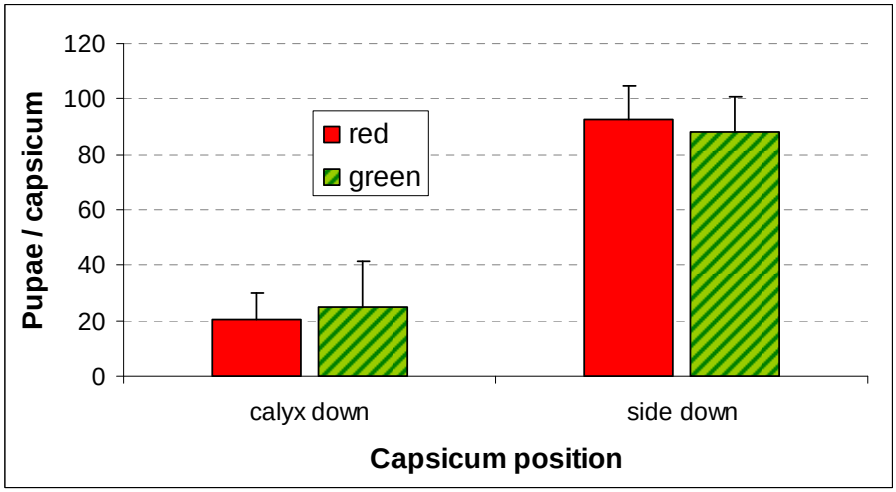

Figure 2.2 - Mean number of pupae recovered per capsicum fruit from red and green capsicums placed either calyx down (n=6x2) or on their side (n=6x3). Bars indicate the standard error of each mean value.

The skin of capsicums may be relatively difficult for flies to penetrate because capsicum fruits, like other Solanaceae, do not have stomata. Without these natural openings, the waxy skin presents an important barrier to oviposition, although clearly not an impenetrable one.

As a result of these trials, it is concluded that the capsicum colour does not affect either the attraction of the flies to the fruit (given a choice between the two) or the number of viable pupae raised from the two ripeness stages. Subsequent trials therefore did not include red or green colour as a factor.

2.4 Key Points

- Differences between green and red capsicum fruit in terms of their attractiveness and suitability for Qfly oviposition were examined using a series of “choice” tests.
- Enough mature female Qflies to potentially lay 1 egg/g fruit were introduced into cages containing 1 red and 1 green capsicum fruit.
- After 24 hours capsicums were removed and incubated individually to allow larvae to develop and pupate.

- Blocking easy access to the calyx area reduced pupal recovery, suggesting the waxy cuticle of capsicums acts as a barrier to oviposition.

- There was no effect of capsicum colour on the number of pupae recovered.

- It is concluded that green and red capsicums are equally attractive to Qfly and suitable for larval development.

3 Cold tolerance of different Qfly lifestages, with and without a hot water shower

3.1 Introduction

The first step in developing a disinfestation protocol for a fruit or vegetable is the determination of the lifestage of the target pest which is most tolerant to the proposed treatment. Subsequent large scale trials can then be done on this lifestage only. This data can also be used to estimate the minimum treatment which will result in no survivors in large scale trials.

In this case, it was important to determine not only the Qfly lifestage which was most tolerant to cold storage in *Capsicum annuum* fruit, but whether this was affected by variety and fruit characteristics. It was also necessary to determine whether survival was increased, decreased or unaffected by a short hot water shower treatment.

A series of trials were conducted examining the effect of storage time at 3^{o}C on mortality of eggs, 1st, 2nd and 3rd instar Qfly larvae in 3 different varieties of *C. annuum*. The varieties were chosen to reflect the span of commercial cultivars, ranging from large and sweet (capsicums) to medium size with moderate heat (cayenne chillies) down to small and extremely hot (birdseye chillies).

The trials were then repeated with the addition of a hot water shower treatment before storage. This was to reflect what would likely happen during commercial practice; harvested and potentially infested fruit would be processed through a packing line, then packed and immediately cold stored ready for shipping.

3.2 Methods

3.2.1 Fruit supply

Disinfestation trials must be conducted using fruit which is completely free of insecticides. This ensures that larvae survive and develop normally in the fruit, and that the effects of the treatment are not confounded by other factors.

In the case of capsicums and chillies, obtaining a regular supply of unsprayed fruit was problematic. At the time of the trial commencement, all fruit from Qld was insecticide treated before transport. Even if this could be avoided, growing a crop of capsicums or chillies without protective insecticides in the high pest pressure areas of Bundaberg and Bowen proved difficult to impossible; Austchilli kept a small area of their chilli crop free of all insecticides for use in the trial. However, some of these chillies arrived at the laboratory already infested with fruit fly, and the crop was abandoned.

As a result, most of the fruit used in these trials was grown on site, over two seasons, in NSW DPI greenhouses located at Narara. The greenhouses were fully enclosed up to the roof vents, with double doors to ensure no insects were able to enter from ground level. Although the roof vents could potentially allow incursion, Qflies have not been found to enter greenhouses through this route – the height of the vents (>3m), combined with lack of visual cues and positive internal pressure is thought to greatly discourage ingress.

Capsicums, red cayenne chillies and birdseye chillies were grown in bags of cocopeat using a run to waste system (Figure 3.1). Plants were grown two to a bag with string supports. Sprays of "Eco-Oil" organic pesticide were used to control whitefly and aphids late in the growing season. A small test carried out on Eco-Oil treated fruit confirmed this did not affect either infestation or larval mortality by Qfly in the laboratory environment (data not shown).

Figure 3.1 - Production of chilli and capsicum fruit for trials in the NSW DPI greenhouse at Narara, showing planting, training of young plants, mature capsicums and chillis and fruit ready to harvest.

After a number of trials had been conducted, the first crop (2010-2011) had to be abandoned due to latent infection of the fruit with what appeared to be Rhizopus sp. This disease was not evident on intact fruit, but appeared within days once fruit were damaged and infested with Qfly (Figure 3.2). As this disease caused flesh breakdown it had a major effect on larval survival rates. Trials were therefore stopped until the second crop matured during the summer of 2011-2012, allowing the completion of all "most treatment tolerant lifestage" tests.

Figure 3.2 - Apparent growth of Rhizopus sp. on Qfly infested capsicum fruit

3.2.2 Fruit infestation

All infestations were conducted using the experimental Qfly colony located at the NSW Department of Primary Industries Laboratory at Ourimbah. The flies are kept at an optimum 26^{o}C with 12 hour day / night cycling including 1 hour simulated dawn and dusk. Adult flies are fed a mixture of protein and sugar, becoming sexually mature after 2 weeks and disposed of after 4 weeks as vigour declines. The colony is replaced with wild stock at least every two years.

Fruit were picked early in the morning and immediately taken to the Ourimbah laboratory (approx 15minute journey). Each trial used 8-9kg of red cayenne chillies, 6-7kg of birdseye chillies, 160 small capsicums or 100 large capsicums.

To infest, the fruit were spiked with a standardised wooden block with fine protruding needles, then placed on top of cages containing 2-4 week old Qflies (Figure 3.3). This allowed the female flies to lay eggs directly into the fruit. It was noted that the uneven shape of many capsicums and chillies resulted in only a small contact surface with the cage. To ensure that flies could potentially access every fruit, fruit were rotated half way through the infestation (approx 1 hour). Both capsicum and chillies are highly attractive to Qflies, and large numbers of eggs were generally laid (Figure 3.4).

Figure 3.3 - Chillies placed on cages of mature Qflies at the Ourimbah laboratory, allowing females to lay eggs directly into the fruit.

Figure 3.4 - Qflies laying eggs into chillies (L) and capsicum (R)

3.2.3 Fruit treatments

After approximately 2 hours, fruit were removed from the Qfly cages. In the case of chillies, infested fruit were weighed and packed into mesh bags containing 150g (birdseye) or 200g (cayenne), with fruit from each cage included in each bag. The bags were then randomly allocated into 5 treatment groups (total = 40-45 bags). In the case of capsicums, individual fruit from each cage were allocated randomly to 5

treatment groups. In all cases this was done to reduce potential variability caused by differences among the cages.

The groups were designated; controls (no treatment), eggs, 1st instar, 2nd instar and 3rd instar larvae. A few additional fruit were used to monitor larval development.

Controls were placed directly into tubs containing a small amount of vermiculite. These were lidded with terylene gauze to permit aeration and incubated at 26°C to allow eggs to hatch and larvae to develop and pupate (Figure 3.6). After 10 days and again after 17 days the vermiculite was sieved to remove pupae. The total pupae from each unit were counted. The average number of pupae recovered, ± the standard error of the mean, was used to estimate the number of pupae per fruit (capsicums) or per gram (chillies).

The remaining fruit were incubated at 26°C until at least 70% of live larvae reached the target maturity. This was assessed by gently washing the flesh of 3 individual fruit through a series of soil sieves. The number of eggs and larvae at each lifestage were counted using a light microscope. This generally occurred after 1 day (eggs), 2 days (1st instar), 3 days (2nd instar) and 5 days (3rd instar).

Once target maturity was reached cold disinfestation could commence. The infested samples were either placed directly into 3°C storage, or subjected to a short heat treatment before storage as previously.

The hot water shower (HWS) was constructed using a hot water bath, a pump and a perforated header tank with water delivery area 14cm x 24cm (Figure 3.5). The pump had sufficient flow rate (4.45L.min-1) to maintain a minimum of 3cm head in the fully foam insulated header tank. The rear and sides of the unit were covered with craftboard to provide additional temperature insulation. Water temperature was controlled to within 0.1°C of setpoint with a digital temperature controller (Thermoline Scientific, WiseCircu fuzzy control system). The temperature controller also circulated the water within the main tank. Temperatures in the header tank and at the bottom of the shower drop were measured independently and found to be within 0.1°C of the main tank.

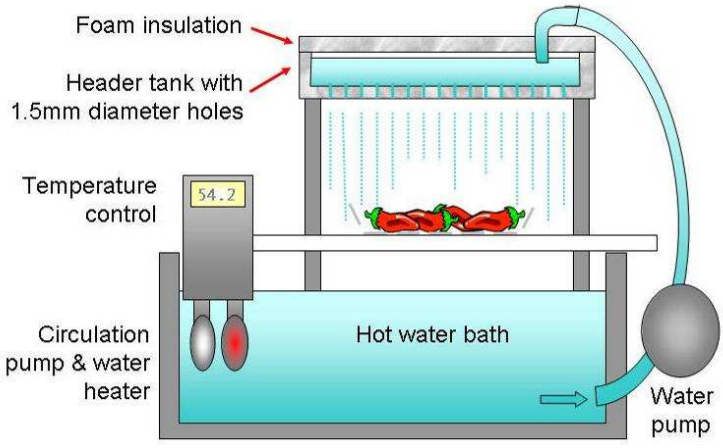

Figure 3.5 - Experimental hot water shower system

Fruit were treated with the HWS at 55°C for 60 seconds. The capsicums were rotated half way through treatment to ensure their whole surface was exposed to the water, while groups of chillies were moved continuously under the centre of the flow (Figure 3.6). After treatment the fruit were roughly dried with paper towel then air-

dried with a fan for several minutes. They were then placed into covered tubs and transferred to 3°C.

Treatments were as follows;

Cold storage only	*Hot water shower + cold storage*
2 days @ 3°C	HWS, no cold storage
3 days @ 3°C	HWS then 2 days @ 3°C
4 days @ 3°C	HWS then 3 days @ 3°C
5 days @ 3°C	HWS then 4 days @ 3°C
6 days @ 3°C	HWS then 5 days @ 3°C
7 days @ 3°C	HWS then 6 days @ 3°C
9 days @ 3°C	HWS then 7 days @ 3°C
11 days @ 3°C	HWS then 9 days @ 3°C
	HWS then 11 days @ 3°C

After removal from cold storage, each group of fruit was incubated at 26°C to allow surviving larvae to develop and pupate. Pupae were counted after 10 and 17 days to estimate mortality.

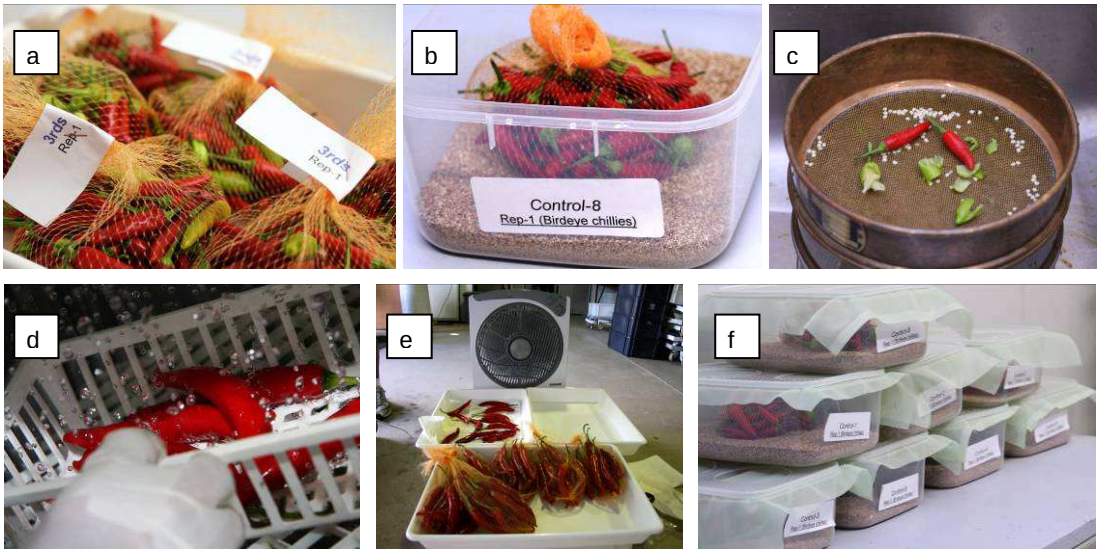

Figure 3.6 - Infested fruit were randomly allocated to 5 groups designated controls, eggs, 1st, 2nd or 3rd instar (a). Controls were placed in containers with vermiculite and stored at 26°C (b). Each day a few additional samples were passed through a series of soil sieves to retrieve eggs and larvae and monitor development (c). Once 70% of larvae reached the target lifestage, the designated treatment units were treated with a hot water shower (d) then cooled (e) or simply placed directly into 3°C storage. After 1-11 days at 3°C individual samples were transferred to 26°C to allow surviving larvae to develop and pupate (f).

At completion of each storage time one bag of chillies or 3 capsicums were transferred from 3°C storage to ventilated tubs at 26°C. This allowed any surviving larvae to develop and pupate. Vermiculite was sieved 7 and 14 days after removal to determine pupation, as described for controls.

The whole experiment was replicated over time for capsicums, birdseye chillies and cayenne chillies. Between three – five replications were conducted so as to ensure that a total of at least 1,500 insects were treated for each product + lifestage + storage time combination (Table 3.1). This would provide sufficient data for confidence in the overall conclusions. The experimental procedure is summarised in Figure 3.7.

Table 3.1 - Number of replications of each "most treatment tolerant lifestage" test

	Number of Replications	
	Cold only	*Cold + HWS*
Capsicum	5	4
Chilli – cayenne	3	3
Chilli - birdseye	3	- *

** - Tests using birdseye chillies were not repeated with the hot water shower as data from cold storage only indicated that they did not differ from the red cayenne variety.*

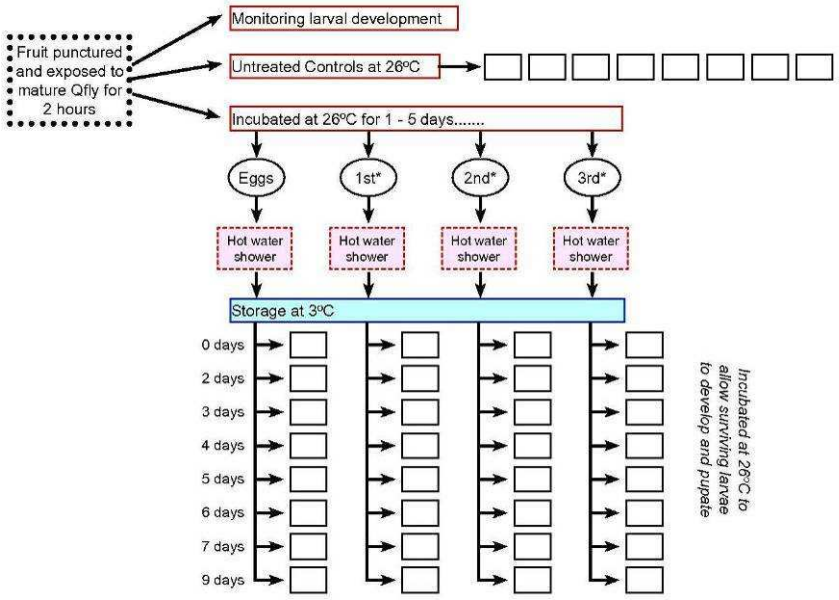

Figure 3.7 - Experimental design for "most treatment tolerant lifestage" tests. Some samples were treated with a HWS for 60 seconds at 55°C, others were placed directly into cold storage for up to 11 days.

3.2.4 Statistical analysis

The mean number of larvae per capsicum / per gram of chillies was estimated using the pupae recovered from the untreated controls. This was used to calculate the percentage mortality for each treatment and lifestage combination.

Qfly survival data was further analysed separately for each fruit type and treatment. GenStat (VSNI 2011) code was prepared using probit analysis methodology which specifically included analysis of replicates over time. The robustness of the model was increased by including the observed variability in mean Qfly pupae/fruit from one infestation event to the next.

Significant heterogeneity was identified using a χ^2 test of residual deviance. When differences between infestation events were significant (5% level), the variance of the estimated parameters was scaled by the corresponding heterogeneity factor equal to the residual mean deviance (Finney, 1971). A range of lethal number of days in cold storage plus the associated 95% confidence intervals were calculated using the method described by Robertson *et al*. (2007) (LD50 , LD90, LD99).

Initial analysis of the results suggested that 1st instar larvae were the most cold tolerant. To test whether this was true, the resistance of eggs, 2nd and 3rd instar larvae to cold storage was compared to the resistance of 1st instars for each vegetable / treatment combination. If the upper 95% confidence interval value was<1, there was a significant difference in cold susceptibility between the instars.

3.3 Results and Discussion

3.3.1 Infestation rates

The number of larvae per fruit (LPF) varied between infestation dates. Despite using a standard method to infest the fruit on every occasion, and compiling fruit from different fruit fly cages for each treatment unit, the number of LPF varied more than ten-fold (Table 3.2).

Table 3.2 - The minimum, maximum and mean number of pupae recovered from each replicate group of capsicum fruit or each 10 grams of chilli fruit, expressed as a mean ± the standard error from all replications (n=3-9), as well as the estimated total number of larvae treated for each combination of cold storage time, larval development stage and HWS / cold storage only

	Pupae recovered per capsicum *or* per 10g chillies			**Total treated larvae per treatment (est)**	
	Minimum	*Maximum*	*Mean*	*Cold only*	*HWS + cold*
Capsicum	60 ± 22	320 ± 79	204 ± 51	1662	1767
Chilli – cayenne	28 ± 8	53 ± 15	41 ± 12	2837	1981
Chilli - birdseye	9 ± 2	37 ± 18	23 ± 11	1022	-

Variability between infestation events is a common issue in conducting this type of research with fruit flies. While this variation reflects what almost certainly happens in the orchard, the reasons for such effects in a controlled laboratory environment are not well understood. Small variations in weather, colony fitness, fruit attributes or simply the way the fruit was placed on the cage for infestation may either stimulate or inhibit egg laying and larval survival. The issue is not unique to Qfly; Fallik *et al*. (2012) also reported significant variability in pupal recovery of Medfly from capsicums, despite artificially infesting the fruit with a known volume of eggs to reduce this effect.

3.3.2 Effect of time and larval development stage on thermal tolerance

Full mortality data for all replications are included in Appendix 1 of this report.

In most cases, only 2-4 days at 3°C was sufficient to kill around 50% of all lifestages in all tested varieties (Table 3.3). For all lifestages other than 1st instars, 4-5 days at 3°C resulted in 90% mortality. No survivors were found when fruit was stored for more than 7 days at 3°C, regardless of lifestage or cultivar.

Table 3.3 - Storage time at 3°C estimated to result in 50% (LD50), 90% (LD90) and 99% (LD99) mortality of eggs, 1st, 2nd and 3rd instar Qfly larvae in birdseye chillies, cayenne chillies and capsicums, including the lower and upper 95% confidence limits (LCL, UCL). All values calculated using Probit analysis.

	Lifestage	LD50			LD90			LD99		
		Days	*LCL*	*UCL*	*Days*	*LCL*	*UCL*	*Days*	*LCL*	*UCL*
Birdseye Chillies	eggs	2.2	1.0	3.0	3.8	2.7	4.5	5.9	5.2	6.9
	1st	3.6	2.6	4.2	5.9	5.2	6.7	8.9	7.5	12.1
	2nd	1.8	0.2	3.1	3.4	1.3	4.5	5.7	4.5	7.4
	3rd	1.2	0.2	2.0	2.5	0.9	3.4	4.6	3.3	5.3
Cayenne Chillies	eggs	2.2	1.4	2.8	3.3	2.6	3.9	4.8	4.2	5.3
	1st	3.8	2.8	4.4	5.7	5.0	6.5	8.0	6.9	10.2
	2nd	2.1	1.0	2.8	4.0	3.0	4.8	6.9	5.9	8.6
	3rd	2.9	2.5	3.2	4.5	4.1	4.8	6.3	6.0	6.7
Capsicum	eggs	2.8	1.9	3.4	4.4	3.7	5.0	6.5	5.9	7.5
	1st	3.6	2.9	4.1	6.9	6.1	8.1	11.9	9.9	15.9
	2nd	2.5	1.8	3.0	4.0	3.4	4.5	5.8	5.4	6.4
	3rd	2.4	1.7	2.8	4.2	3.6	4.6	6.6	6.1	7.4

Larval mortality for each lifestage and storage time appeared to be independent of *C. annuum* variety, being similar for birdseye chillies, cayenne chillies and capsicums (Figure 3.8). Subsequent trials therefore used only cayenne chillies and capsicums.

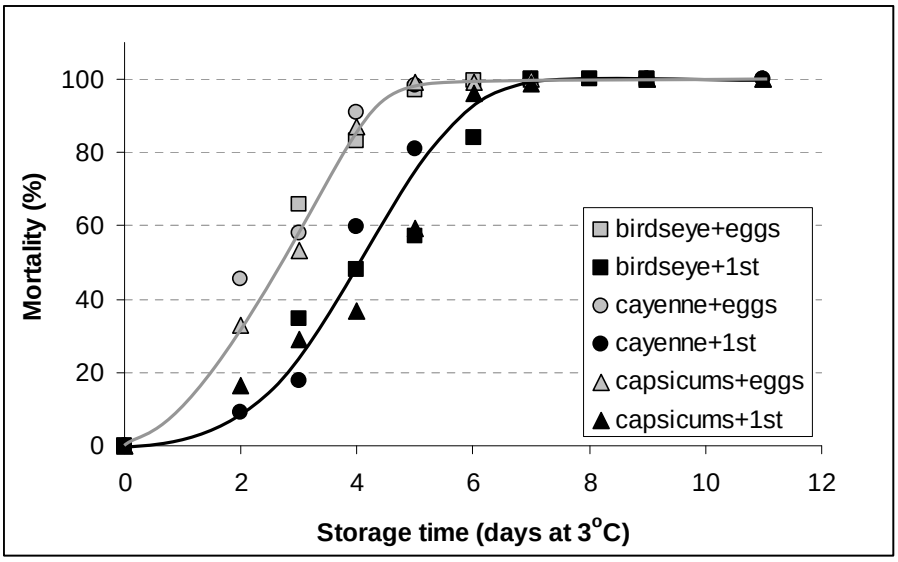

Figure 3.8 - Mean mortality of eggs and 1st instar larvae in birdseye chillies, cayenne chillies and capsicums following storage for 2 – 11 days at 3°C (n=3-5). Data for 2nd and 3rd instar larvae is not shown, but was generally intermediate.

In almost all cases, 1st instar larvae proved more resistant to cold storage than other lifestages (Table 3.4). Examples where this was not the case include achieving 50% mortality of eggs in capsicums and 99% mortality of 2nd instars in chillies, but only at the upper 95% confidence interval in both cases. It was therefore concluded that 1st instar larvae were significantly more treatment tolerant.

Table 3.4 - Relative resistance of 1st instar larvae to cold storage in comparison to other lifestages, including the lower and upper 95% confidence limits (LCL, UCL). Values are compared using the duration of cold treatment required to result in 50% (LD50), 90% (LD90) or 99% (LD99) mortality in either cayenne chillies or capsicums. Values <1 indicate that 1st instar larvae had to be stored for significantly longer to achieve the target mortality.

	Lifestage (*compared to 1st instar*)	**LD50**			**LD90**			**LD99**		
		resist	*LCL*	*UCL*	*resist*	*LCL*	*UCL*	*resist*	*LCL*	*UCL*
Cayenne Chilli	eggs	0.57	0.40	0.82	0.59	0.47	0.73	0.60	0.49	0.73
	2nd	0.55	0.35	0.86	0.71	0.56	0.89	0.87	0.69	1.09
	3rd	0.77	0.61	0.96	0.78	0.68	0.89	0.79	0.68	0.90
Capsicum	eggs	0.76	0.57	1.02	0.63	0.52	0.76	0.54	0.42	0.69
	2nd	0.69	0.52	0.92	0.57	0.48	0.69	0.49	0.39	0.63
	3rd	0.65	0.48	0.87	0.60	0.50	0.72	0.57	0.44	0.73

These results are consistent with previous work demonstrating that 1st instar Qfly larvae can survive longer at low temperatures than other lifestages (Jessup *et al*. 1993, 1998, Heather *et al*. 1996).

3.3.3 Effect of a hot water shower on larval cold tolerance

The hot water shower (HWS) alone caused significant mortality, especially to older larvae (Table 3.5). It might be expected that in a batch of naturally infested fruit, given that all ages of larvae could be present, the HWS would kill at least 50% of Qfly eggs and larvae.

At short storage periods, using the HWS resulted in higher mortality than cold storage alone. However, the effect was lost when storage time increased to 5 days or more, with small numbers of survivors regardless of HWS treatment (Figure 3.9).

Table 3.5 - Mean mortality due to a 60second shower under 55°C water only (no cold storage) for different Qfly lifestages in cayenne chillies and capsicums.

	Mortality (%) due to HWS	
	Cayenne chilli	*Capsicum*
eggs	38.9	37.6
1st	45.4	50.2
2nd	59.7	61.4
3rd	78.9	67.6

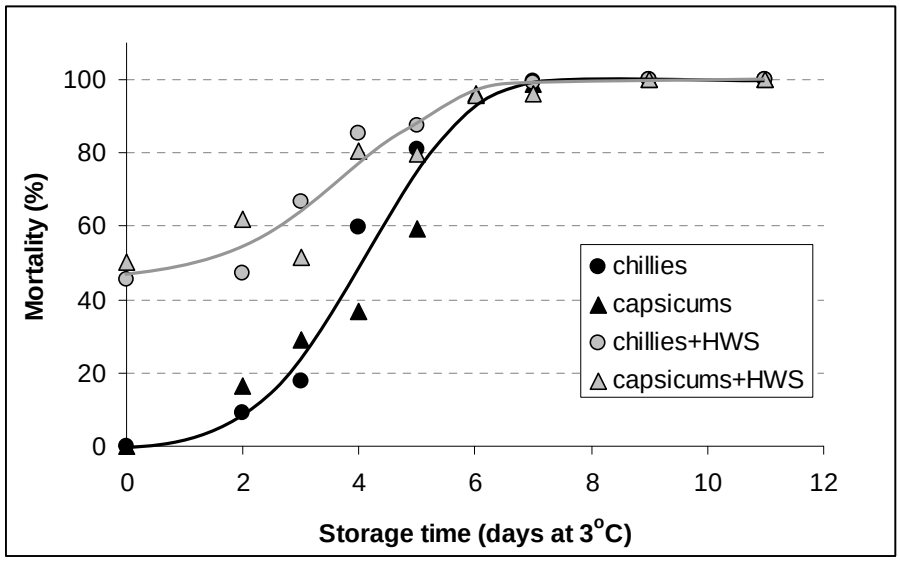

Figure 3.9 - Mean mortality of 1st instar larvae in cayenne chillies and capsicums following a short hot water shower treatment and storage for 2 – 11 days at 3°C, or storage at 3°C only (n=3-5). Data for other instars was similar.

The HWS reduced the storage times required to reach LD50, LD90 or LD99 (Table 3.6). This was due in part to the improved consistency of results, which meant that the data conformed more closely to calculated dose:mortality curves. In particular, it is notable that including the HWS reduced the LD99 storage time for 1st instar larvae in capsicum from 11.9 days to 9.6 days. This was not due to reduced survival at storage times >5days after HWS treatment, but rather to the highly variable nature of the data recorded for non-HWS treated fruit at short storage times.

No pupae were recovered from either chillies or capsicums stored for more than 7 days at 3°C following a HWS. The cold storage time required for effective disinfestation was therefore unaffected by HWS treatment.

Table 3.6 - Relative change in estimated number of days at 3°C required to reach 50%, 90% and 99% mortality when fruit was treated with a HWS+cold compared to fruit subjected to cold storage alone

	Lifestage	**Change in days @ 3°C needed to reach target mortality**		
		LD50	*LD90*	*LD99*
Cayenne Chillies	eggs	+0.1	-0.9	-2.3
	1st	-1.4	-1.0	-0.1
	2nd	-0.7	-1.2	-1.7
	3rd	-1.5	-1.2	-0.2
Capsicum	eggs	-1.5	-1.6	-1.2
	1st	-1.4	-1.9	-2.3
	2nd	-1.5	-1.1	-1.2
	3rd	-1.2	-1.2	-1.5

In this trial, the HWS treatment did not increase the cold tolerance of the larvae. In most cases, HWS actually enhanced mortality due to cold storage, especially of more mature lifestages after short storage times. This is consistent with the results of Jang

et al., (2001), who claimed that a heat shock of 38^{o}C did not increase survival of Mediterranean fruit fly (*Ceratitis capitata*) in avocadoes. Fallik *et a*l., (2012) demonstrated that 21 days at 1.5^{o}C is sufficient to disinfest capsicums from all lifestages of *C. capitata*, regardless of whether or not fruit are subjected to a 15 second hot water rinse and brush at 55^{o}C before treatment. It is therefore concluded that although adding a HWS before storage cannot replace cold disinfestation, it will enhance the effectiveness of a subsequent cold storage treatment.

3.4 Key Points

- The effect of 3^{o}C storage on mortality of eggs, 1st instar, 2nd instar and 3rd instar Qfly larvae was tested in 3 commercial varieties of *Capsicum annuum*; capsicums, cayenne chillies and birdseye chillies
- The results were compared to parallel trials where fruit was treated with a 60 second shower under 55^{o}C water (HWS) just before storage
- 1st instar larvae were found to be the most cold tolerant for all hosts and regardless of HWS treatment
- The HWS alone resulted in 39-79% mean mortality, with the higher values achieved for more mature larvae. It is estimated that at least 50% of a natural population would be killed by a HWS treatment.
- The HWS reduced the storage time required to achieve LD50, LD90 and LD99. Larvae became slightly less, not more, cold tolerant as a result of HWS treatment.
- No pupae were recovered from any fruit stored for more than 7 days at 3^{o}C.
- Based on these results, a cold storage period of 10 days is likely to provide a very high level of quarantine security against Qfly.

4 Disinfestation of *C. annuum* varieties from Queensland fruit fly – large scale trials

4.1 Introduction

The series of trials reported in Section 3 of this report clearly showed that 1st instar larvae were significantly more cold tolerant than eggs or mature lifestages of Qfly. This was independent of whether or not cold was combined with a hot water shower (HWS) treatment, or if the insects were infesting birdseye chillies, cayenne chillies or capsicums.

With the exception of 1st instar larvae in cold stored capsicums (for which results at short storage times were highly variable), LD99 values calculated through probit analysis ranged from 7.9 days to 9.6 days at 3°C. Despite this, no pupae were recovered from any fruit that was stored for more than 7 days at 3°C. It was therefore decided that 10 days at 3°C should be enough to provide ample quarantine security against all stages of Qfly in cultivars of *C. annuum*.

4.2 Method

4.2.1 Fruit supply

As previously, 'block' type capsicums and cayenne chillies used in the large scale trials were grown in protected greenhouses located at Narara, NSW. No pesticides (other than EcoOil) were used on the crop so as to ensure that all fruit were suitable for Qfly infestation and development.

Although double doors and mesh sides were used to block entry of Qfly and other pests, flies could potentially have entered through open roof vents. No Qfly were detected inside the greenhouses or infesting the fruit during the trials, possibly due to the lack of visual cues, height of the vents and positive pressure inside the house.

Trials were conducted over two seasons (2010-2011 and 2011-2012) using both red and green fruit.

4.2.2 Fruit infestation

As previously described, all infestations were conducted using the experimental Qfly colony located at the NSW Department of Primary Industries Laboratory at Ourimbah. The flies are kept at an optimum 26°C with 12 hour day / night cycling including 1 hour simulated dawn and dusk. Adult flies are fed a mixture of protein and sugar, becoming sexually mature after 2 weeks and disposed of after 4 weeks as vigour declines. The colony is replaced with wild stock at least every two years.

Fruit were picked early in the morning and immediately taken to the Ourimbah laboratory. Each trial used slightly over 8kg of red cayenne chillies or 300 large capsicums.

To infest, the fruit were spiked with a standardised wooden block with fine protruding needles, then placed on top of 4-5 cages containing mature Qflies. To ensure that

flies could potentially access every fruit, fruit were rotated half way through the infestation (approx 1 hour).

After approximately 2 hours, the fruit on each cage was collected and placed in individual bins. Treatment units of 200g cayenne chillies or 6 capsicums were assembled using fruit randomly selected from each of the bins. This minimised any variation due to differences between cages of Qflies. A few additional chillies and capsicums were kept aside for use monitoring larval development.

4.2.3 Temperature recording

Fruit core and air temperatures were monitored using Squirrel temperature data loggers (Model 2040, Grant Instruments, UK). The probes used were U Thermistor types, which have a range of -50^{o}C to 150^{o}C and are accurate to within 0.2^{o}C.

The probes were calibrated by immersion in a slurry of melting ice made from distilled water. The temperature of this mix was checked using a reference thermometer and found to be 0^{o}C. Temperatures were logged every 30 seconds. Once values stabilised (>3 consecutive readings the same) these were used as calibration values for the trial

Figure 4.1 - Calibration of the temperature probes in a slurry of melting ice made from distilled water

The squirrel was set to record temperature every 6 minutes (0.1 hours) during all trials. Results were downloaded for analysis with Excel.

4.2.4 Fruit treatments

A total of 40 bags of chillies or 50 bags of capsicums were used for each trial. Ten bagged treatment units were allocated to use as untreated controls. These fruit were placed directly onto vermiculite inside ventilated plastic tubs and incubated at 26^{o}C to allow larvae to develop and pupate. After 10 and 14 days the vermiculite was sieved and the pupae counted.

The remaining treatment units (30 bags chillies or 40 bags capsicums) were placed inside large plastic tubs and incubated at 26^{o}C until the eggs had hatched and >70% of larvae were 1st instar (Figure 4.2 a-c).

Larval development was monitored as previously, with 2-3 individual capsicums or chillies assessed daily by gently washing the eggs, larvae and fruit flesh through a series of soil sieves. Eggs and larvae were examined under a light microscope against a black plate, mouthparts were used to determine development and the number of each lifestage recorded.

Once the trial was ready to start, the bags were either HWS treated as previously (60 seconds under 55°C) or simply pre-cooled, ready for loading into the 3°C coolroom. Pre-cooling was done to reduce the time required for fruit temperatures to fall from ~55°C or 26°C to close to 3°C, as this would potentially allow further larval development. Open tubs with bagged fruit were placed in a single layer in a cool room set at 3°C. Internal temperatures monitored with a hand held probe until they fell to around 6°C. This generally took an hour or more.

The coolroom used during the trial already contained pre-cooled pallets of citrus – these were used as filler fruit. To load, some citrus was removed from each carton and 2-3 bags of infested capsicums or chillies added. Temperature probes were inserted into fruit in seven of the cartons and secured using duct tape (Figure 4.2 d-e). The eighth temperature probe was used to monitor air temperature.

The cartons containing infested fruit were numbered and loaded into random positions on the pallet (Figure 4.2 f). An example of stacking pattern is shown in Figure 4.3.

Figure 4.2 - After infestation (a), fruit were divided into treatment units of 6 capsicums or 200g chillies (b). After randomly selecting 10 bags as untreated controls, the remainder were incubated at 26°C until >70% of larvae were 1st instar (c). Temperature probes were inserted into 7 fruit (d) and the bags loaded into cartons packed with citrus filler fruit (e). Cartons containing infested fruit were labelled and randomly allocated to various positions within the pallet (f).

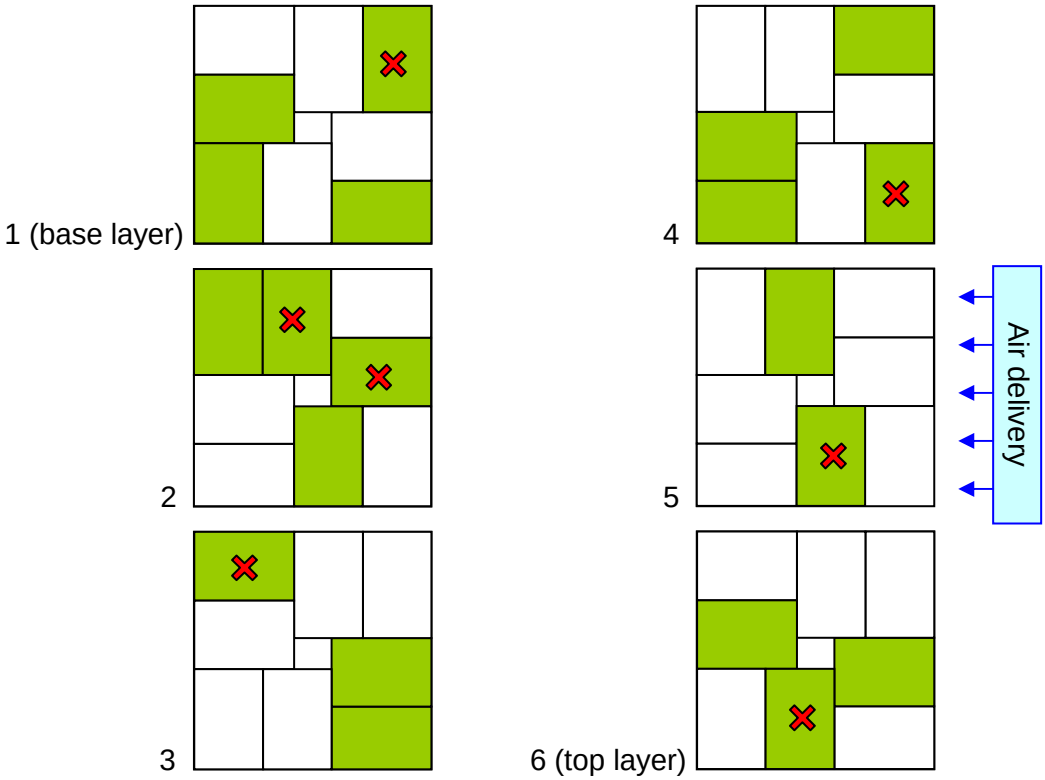

Figure 4.3 - Example of pallet layout. Green boxes indicate cartons containing infested fruit samples, white boxes are cartons with filler fruit only, red crosses indicate cartons with loggers inside.

Cold disinfestation was deemed to have commenced once 5 of the 7 probes fell to 4°C or less. Temperatures were monitored during the trial to ensure that they remained as close as possible to 3°C. After a maximum of 10 days (240 hours) the room was opened, probes retrieved, and all infested fruit samples transferred to 26°C. The samples were placed on mesh inserts in tubs containing a small amount of vermiculite (Figure 4.4). The tubs were covered with insect proof mesh and incubated at 26°C to allow any surviving larvae to develop and pupate. After 10 days the vermiculite was sieved to remove any pupae.

Figure 4.4 - Treated fruit were incubated over vermiculite at 26°C to allow any surviving larvae to develop and pupate

A total of eight separate large scale trials were conducted; four with capsicums and four with cayenne chillies. Of these, the first three replications used cold treatment alone. On the fourth replication, the infested fruit were exposed to a HWS just prior to disinfestation.

4.3 Results

4.3.1 Fruit temperatures

The room was kept sealed for the duration of treatment, with air temperatures primarily ranging between 2.4 – 4°C. Once the setpoint had been reached, capsicum and chilli flesh temperatures remained very stable, generally varying by <0.2°C (Figure 4.5).

During initial trials with capsicums one or more probes fell below 2.9°C. Subsequently, room temperatures were increased slightly so that all probes remained at 2.9°C or higher for the duration of treatment, this being the limit of accuracy of the probes. A summary of all of the temperatures recorded during the trials is included in Appendix 2.

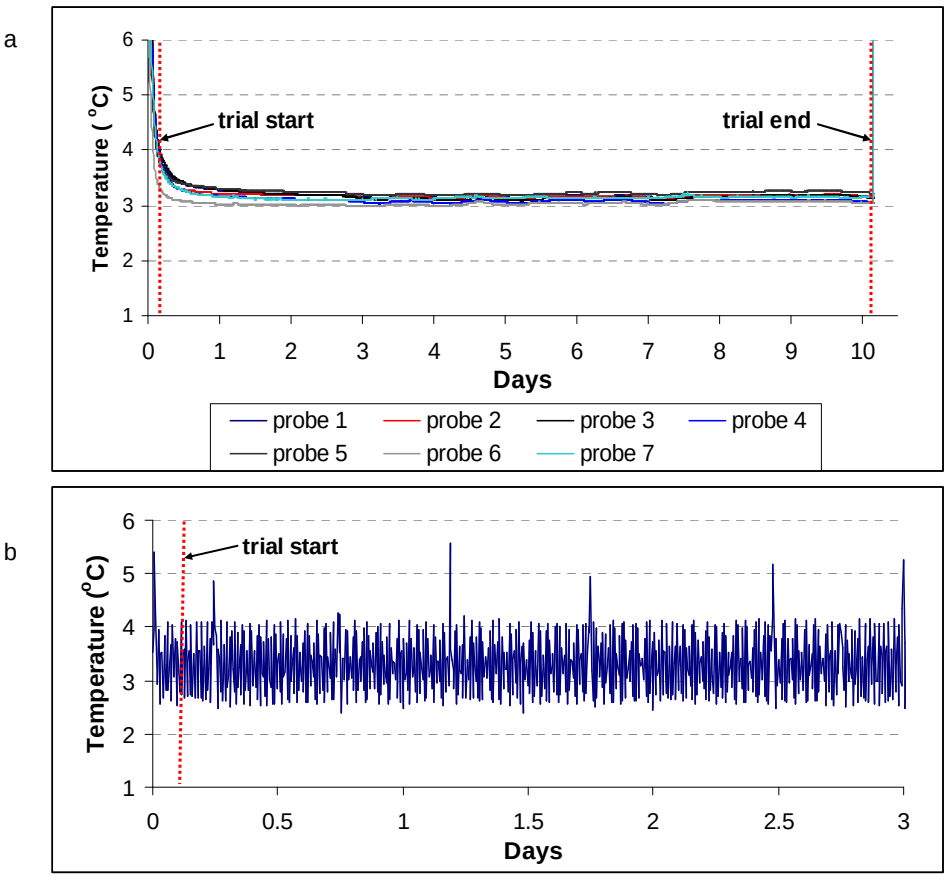

Figure 4.5 - Internal flesh temperatures (a) and room air temperatures (b) recorded during 10 day disinfestation treatment. Example is from replicate 3, chillies.

4.3.2 Insect mortality

The majority of eggs had hatched two days after the original infestation. Development rates were highly consistent, with 80-90% of eggs and larvae at 1st instar lifestage after this time. This meant that a high proportion of the total larvae in the fruit were 1st instar at the time treatment commenced (Table 4.1).

Consistently high numbers of pupae were recovered from the untreated controls for both capsicums and chillies. Across all trials, pupae per capsicum fruit averaged 157 ± 10 while pupae per 10g chillies averaged 52 ± 6.

Based on these values and taking the lower confidence interval for average infestation rates, it was estimated that at least 80,148 and 91,363 1st instar larvae were treated in capsicums and chillies respectively, giving an total of 171,511 (Table 4.1).

Table 4.1 - Estimate of minimum and maximum number of 1st instar larvae killed during the trial, based on the percentage of all larvae which were 1st instars at the trial commencement, multiplied by the number of pupae per capsicum or per 10g chillies in the controls and the total number of fruit subjected to a cold disinfestation treatment (6kg chillies or 240 capsicums). The low and upper 95% confidence intervals (LCL, UCL)were calculated by multiplying the standard error of the mean by 1.96.

	Replicate	Percentage 1st instars at trial start	Pupae / cap *OR* 10g chilli (controls)	1st instar larvae / cap *OR* 10g chilli (est)	Total 1st instar larvae killed by cold treatment	
					LCL	*UCL*
Capsicum	1	80.7	126.1	101.7	18,954	29,879
	2	81.2	147.6	119.8	22,214	35,302
	3	82.3	230.9	187.5	38,980	51,009
	4 (HWS)	81.2	122.9	99.8	19,822	28,080
				TOTAL	*80,148*	*144,270*
Chillies	1	82.3	23.74	19.5	10,212	13,233
	2	85.9	39.9	34.3	18,504	22,609
	3	85.9	106.9	91.8	45,571	64,601
	4 (HWS)	86.7	37.9	32.6	17,076	22,017
				TOTAL	*91,363*	*122,460*
				GRAND TOTAL	***171,511***	***266,730***

****No pupae were recovered from any of the cold stored fruit****

Probit 9 mortality is equivalent to 93,750 insects treated with no survivors (Corcoran, 1993). As the total number of insects killed by 10 days at 3°C or higher exceeded 171,000, this series of results provides better than Probit 9 evidence of treatment efficacy.

4.4 Key Points

- A series of 8 large scale disinfestation trials were conducted, half with capsicums and half with chillies. The 4th replicate trial for each variety was subjected to a HWS immediately prior to treatment commencing.
- Capsicums and chillies infested with 1st instar larvae were packed inside cartons of filler fruit and randomly distributed within a pallet in the 3°C treatment room.
- Temperatures averaged 3°C or higher for all trials. Flesh temperatures remained very stable during treatment.

- Large numbers of pupae were recovered from untreated control fruit, averaging 157 ± 10 for capsicums and 52 ± 6 per 10g chillies.

- There were no survivors from any of the capsicums and chillies stored for 10 days at 3^{o}C.

- At the lower 95% confidence interval, it was estimated that at least 171,511 1st instar larvae were treated, suggesting that this treatment provides greater than Probit 9 quarantine security against Qfly.

5 Application of the HWS under commercial conditions

5.1 Introduction

So far, trials had demonstrated that;

1. A hot water shower (HWS) at 55^{o}C for 60 seconds can protect chilling susceptible capsicums and chillies from damage during storage at 3^{o}C
2. A HWS increases, rather than decreases subsequent Qfly mortality during storage at 3^{o}C
3. Ten days at 3^{o}C can provide a very high level of quarantine security against Qfly infestation

While the experimental evidence appeared sound, applying this method in a commercial environment could well present new challenges.

The original idea of testing a HWS treatment had come about from observing existing packing-lines which, at that time, commonly applied a dimethoate drench to fruit immediately after harvest. Dimethoate is no longer registered for postharvest application to either capsicums or chillies. Re-designing this equipment to use hot water instead of dimethoate seemed likely to be a relatively simple task.

However, the cost and practical implications of heating and maintaining drench water at 55^{o}C needed to be examined. Also, it seemed worth examining whether this system would provide quality benefits even if fruit were simply being marketed through normal channels, and not subjected to cold disinfestation at all.

AustChilli, who had already assisted with earlier parts of the trial work, expressed interest in evaluating the system at their packing line in Bundaberg. They were able to re-engineer some existing equipment to apply the treatment. A trial was therefore developed examining the effects of the HWS on storage characteristics of a variety of different chillies and field grown capsicums.

5.2 Method

5.2.1 Source of fruit

With the exception of the red capsicums, which were not locally available, all fruit was sourced from within the immediate area of the AustChilli facility in Bundaberg. Cartons of red capsicums were purchased from Brisbane markets specifically for the trial. All green capsicums were picked the afternoon before the trial at a local property (Figure 5.1).

In order to have samples of six different varieties of chillies available on the same day for testing, some had been harvested several days before and cool stored. Freshness varied as a result, so although varietal effects were included in the analysis, these may not be due to either the treatment or the variety used. This was unfortunate for the trial, but reflects the commercial reality and difficulties involved in conducting such a trial.

In summary, *C. annuum* varieties selected for testing were;

- Sweet capsicum (green)
- Sweet capsicum (red)
- Birdseye chillies (red)
- Ball chilli (green)
- Ball chilli (red)
- Jalapeno chilli (green)
- Cayenne chilli (green)
- Cayenne chilli (red)

Figure 5.1 - Green capsicums growing in Bundaberg area

5.2.2 Equipment design

To conduct the trial, Austchilli adapted equipment previously used for dimethoate drenching of chillies. This consisted of a long, enclosed tunnel with internal jets along its length. The conveyor speed had been adjusted so as to provide a treatment time of approximately 1 minute. The jets were connected to two large tanks / reservoirs, which were in turn plumbed into a large domestic water heater (Figure 5.2). Water circulated through the reservoirs and heater so as to maintain a temperature of approximately 55°C. Because this was a single trial, used water was not recirculated but allowed to run to waste.

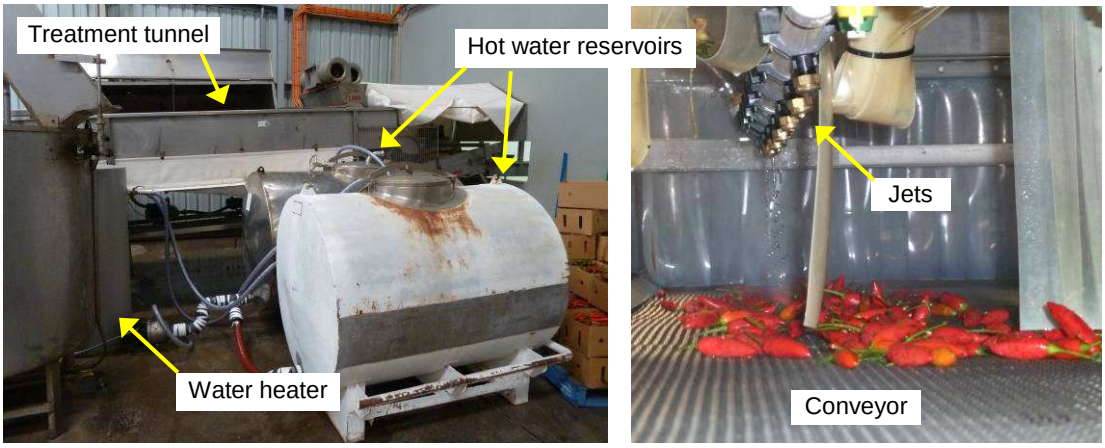

Figure 5.2 - Hot water shower test equipment developed by AustChilli for use in trial

5.2.3 Fruit treatments

The night before the trial the total volume of capsicums / chillies available for testing was divided; one half was stored under ambient conditions inside the packing shed, the remainder were stored inside the cold room at approximately 8°C. These units were further divided and designated replicates 1 and 2; all subsequent activities were conducted independently for each replicate group of chillies or capsicums.

On the morning of the trial each replicate group of fruit was divided into four units. These were allocated to either hot water shower treatment (55°C for approximately 1 minute) *or* control (passed through machine without water running), followed by either a disinfestation treatment (>10 days at 3°C) *or* simulation of normal transport and marketing practices. The trial design is summarised in Figure 5.3. Each treatment unit contained;

Variety	Unit size
Capsicum (green)	10 fruit
Capsicum (red)	5-6 fruit
Birdseye chillies (red)	120-140g
Ball chilli (green)	300-400g
Ball chilli (red)	300-400g
Jalapeno chilli (green)	500-600g
Cayenne chilli (green)	350-450g
Cayenne chilli (red)	350-450g

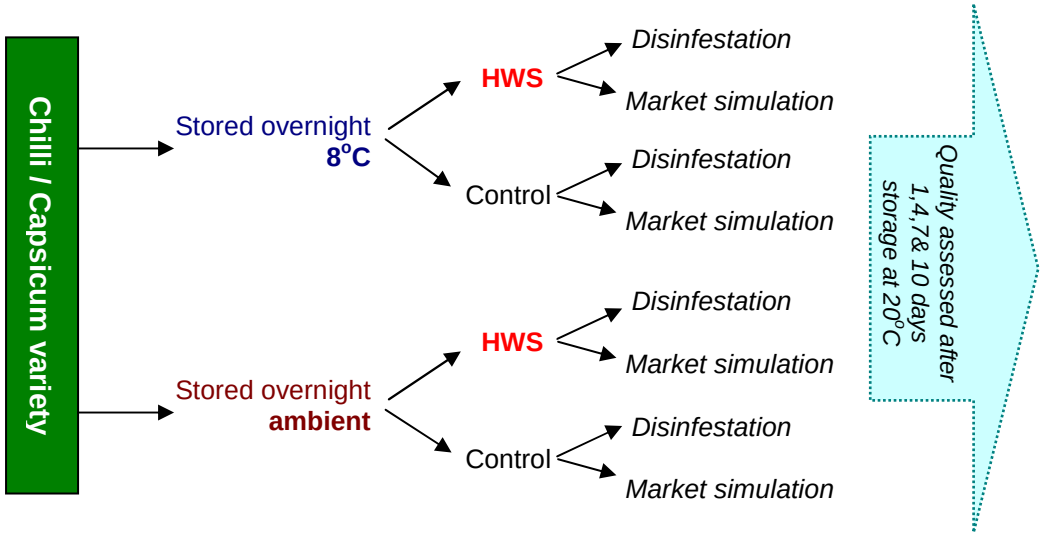

Figure 5.3 - Design of trials conducted at AustChilli, Bundaberg. All treatments (n=8) were replicated in time (n=2) for each variety (n=8). Total treatment units during the trial = 128

All capsicums and chillies were passed through the hot water shower machine. Replicate 1 units were treated first, with the water reheated before treatment of replicate 2. Units designated 'controls' were passed through the unit with the hot water turned off. After processing each unit was collected and allowed to air dry for at least 30 minutes (Figure 5.4). They were then either placed directly into a cardboard box (capsicums) or placed inside an unsealed, labelled plastic bag (chillies) and transferred to the 8°C storage room to await transport.

LogTag data recorders were placed inside eight of the bags and boxes to monitor temperatures during storage and transport. Two additional data recorders were inserted into the capsicums to record internal temperatures. All data loggers recorded temperature to within 0.1^{o}C at 10 minute intervals for the duration of the trial.

Figure 5.4 - Chillies and capsicums were collected and allowed to air dry following hot water shower treatment

One day after treatment all samples were loaded onto a truck for transport to Sydney Markets. They were collected, transported to the DPI laboratory at Ourimbah, and stored for a further 3 days (market simulation) *or* 13 days (disinfestation protocol) before transfer to 20^{o}C for assessment of outturn quality and shelf life.

5.2.4 Quality assessment and analysis

Following removal from cold storage and a day at 20^{o}C, products were weighed and quality was assessed every 3 days during shelf life at 20^{o}C until the samples were graded "unacceptable". Visual grading aids were developed to ensure that, although grading was subjective, assessment was consistent over the period of the trial. The grading scales are shown in Appendix 3.

Each assessment examined calyx and stem browning, rots and acceptability in either 30 randomly selected birdseye chillies, 15 randomly selected chillies of the other varieties or each one of the capsicums in the sample unit. For chillies, the total score for each quality attribute was divided by the number of fruit examined to determine an overall score for the treatment unit. Capsicums were analysed individually.

Results were processed using Microsoft Office Excel 2003 and analysed using CoStat statistical software. Each assessment time was analysed separately to avoid confounding by repeated measures. Factors were analysed individually and as a three way factorial ANOVA to determine differences due to initial temperature, variety, treatment and storage protocol. Mean values were compared using the Student-Newman-Keuls test at the 95% confidence level.

5.3 Results

5.3.1 Temperatures during storage and transport

Although temperature during the hot water shower was monitored, no data could subsequently be extracted from the loggers. Manual checks of water temperature indicated that it ranged from approximately 53 – 58°C during the trial.

One of the issues encountered was the large volume of water required to provide 1 minute of hot water showering. This made it difficult to maintain an even temperature. The aim was to shower the fruit with 12.7L.min^{-1}.1,000cm^{-2}. Actual delivery was closer to 9L.min^{-1} over this area. Despite this, both of the large reservoirs used were exhausted in the time it took to treat the samples. Moreover, it proved difficult to accurately maintain temperature with such a large volume of water.

Fruit temperatures during transport, storage and shelf life are shown in Figure 5.5. During "disinfestation" fruit temperatures ranged from a minimum of 3.2°C to maximum 4.7°C, averaging 3.8 ± 0.15 °C. This was followed by "shelf life" at 20.2 ± 0.3°C. Shelf life temperature averaged 21.1 ± 0.3°C following the shorter period of cold storage included in the "normal marketing" protocol.

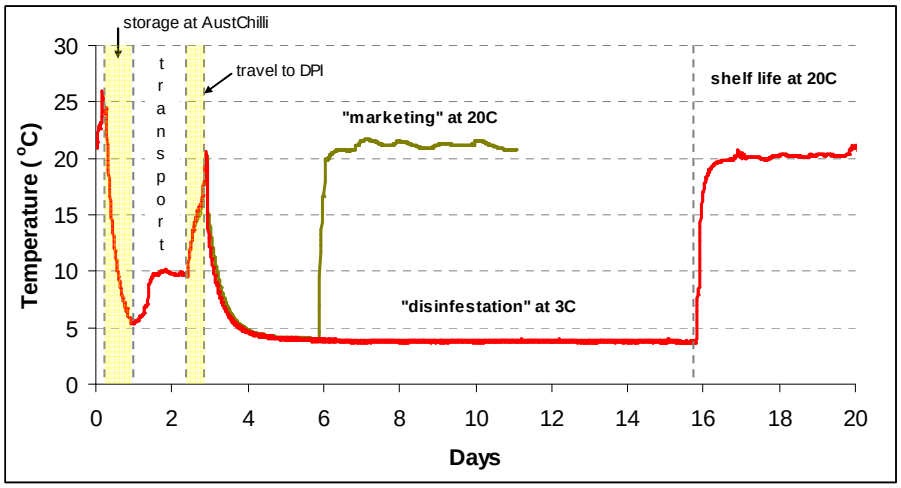

Figure 5.5 - Temperatures during storage, transport and shelf life evaluation of chillies and capsicums transported from AustChilli Bundaberg to the NSW DPI laboratory Ourimbah then subjected to short term cold storage only ("marketing" protocol) or extended cold storage ("disinfestation" protocol). Lines indicate mean values calculated from six LogTag data recorders.

5.3.2 Capsicum quality

Few significant differences were found between any of the treatments. This was particularly the case with the red capsicums, which developed severe rots during storage, regardless of hot water shower (HWS) treatment. These samples had been purchased from Brisbane markets for the trial. It appears likely the fruit had already been stored for some time before the trial commenced.

Increasing the storage time at 3°C ("disinfestation" protocol) increased stem rots and reduced overall acceptability in green capsicums, ($p<0.01$). Although a trend was

observed of the HWS reducing rots and decline in quality, differences were not significant (p=0.35) (Figure 5.6).

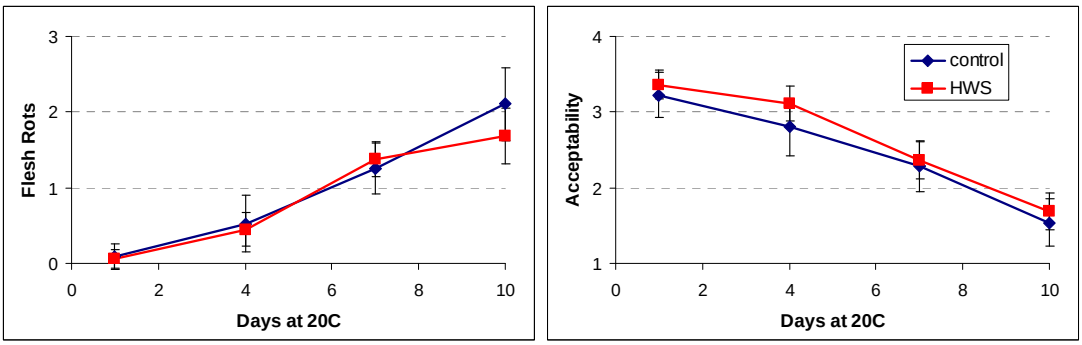

Figure 5.6 - Differences in development of flesh rots and decline in overall acceptability of green capsicums left untreated (control)or exposed to a 60second shower under ~55°C water (HWS) . Points indicate average values calculated from all replications and treatments, bars indicate the standard error of each mean (n=8).

No consistent differences in quality attributes or weight loss were observed between capsicums treated cold compared to those stored at ambient temperatures before the trial, or between the two replicate groups of fruit. Red colour development in green capsicums and stem browning were not affected by any of the treatments tested.

Chilling injury was not observed on either the green or red capsicums, even after extended storage at 3°C. As one of the major effects of a HWS was expected to be reduction of chilling sensitivity, this is a major reason for the minimal differences observed between HWS treated fruit and controls in this trial.

5.3.3 Chilli quality

Perhaps because of the larger number of individual fruit assessed, as well as the consistent quality of the products used, small but significant differences in quality and weight loss were observed between the six different chilli varieties assessed.

5.3.3.1 Variety effects

Following storage and + 4 days shelf life, ball type and birdseye chillies had significantly more stem browning than cayenne and jalapeno types. The ball types were also most likely to develop rots in both the flesh and stem (Figure 5.7).

Such effects are most likely related to the initial condition of the fruit; as the chillies had to be accumulated for several days, the varieties differed in freshness at the start of the test. This conclusion is supported by parallel declines in the percentage of fruit still graded as “marketable” at each assessment. Although the red ball chillies were significantly less acceptable after 4 days at 20°C, the quality of all chilli samples declined at a similar rate during shelf life (Figure 5.7).

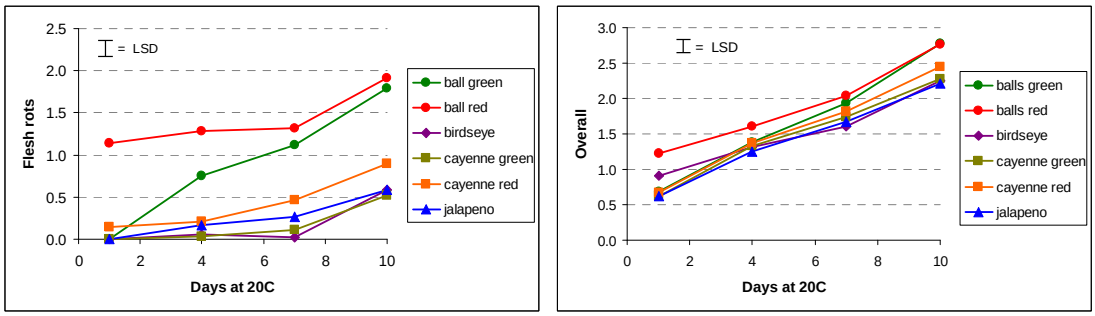

Figure 5.7 - Differences in flesh rots and the percentage of fruit still marketable between different chilli varieties during shelf life at 20°C. Points indicate average values calculated from all replications and treatments (n=16), Bar conservatively indicates the least significant difference between means at each assessment time.

The results of this trial should not be used to suggest that there are differences in shelf life between chilli varieties. Such a conclusion could only be reached using replicated samples of fruit with a well defined production and harvest history.

5.3.3.2 Treatment effects

The initial temperature of the chillies at treatment (cold or ambient) had the least effect on quality of the different treatments tested. However, after 4 days at 20°C, chillies which had been kept cold before treatment were slightly better overall acceptability (p=0.03) than those left under ambient conditions overnight. This reflects a small improvement in the cold chain for these fruit.

Increasing total storage time from 6 to 16 days to disinfest fruit from fruit flies significantly increased stem browning and flesh and stem rots and reduced overall quality (p<0.01). Although differences were not obvious when the chillies were initially removed from storage, they became clear after a single day of shelf life at 20°C (Figure 5.8).

The hot water shower (HWS) slightly mitigated the effects of the increased storage time, reducing rots and browning and improving acceptability (p<0.01). These effects were noted for all chilli varieties tested. Possibly because of the effect on rots, the HWS also reduced weight loss during shelf life (p=0.05), although the effect was small. The greatest effect of the HWS treatment was to significantly increase the percentage of fruit which remained acceptable during shelf life of up to a week, regardless of previous cold storage protocol (p<0.01) (Figure 5.8).

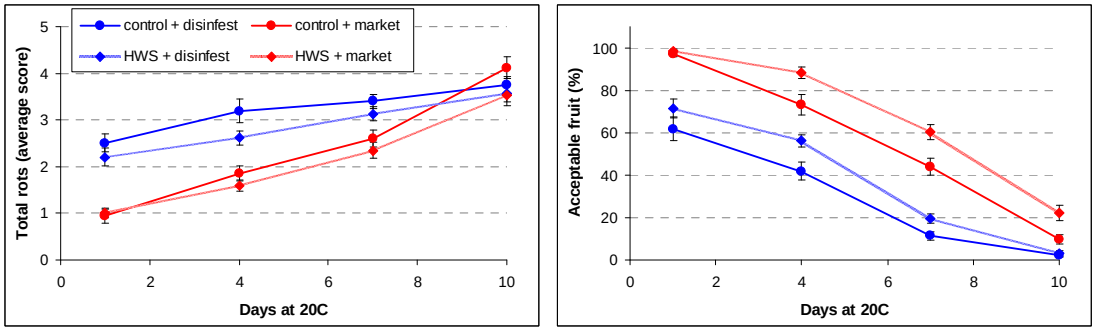

Figure 5.8 - Differences in total rots and the percentage of fruit still marketable during shelf life at 20°C for chillies treated with a hot water shower (HWS) or untreated (control) followed by storage for 6 (market) or 16 (disinfest) days at varying temperatures. Bars indicate the standard error of mean values (n=24).

As with the capsicums, no chilling injury was observed on any of the fruit. As previously noted, one of the major effects of the HWS found previously is to reduce sensitivity to chilling injury, thereby increasing storage life. The fruit used in this trial proved resistant to chilling injury in the times and temperatures tested, reducing the potential benefits of the HWS treatment.

5.4 Discussion

Although significant benefits of using a HWS were found during this trial, most of the effects were relatively small. This is primarily due to the lack of chilling injury observed on all of the samples. Chilling sensitivity can be affected by factors including growing conditions, plant nutrition, variety and delay between harvest and storage. Symptom development is the result of a complex interaction between time and temperature, which is generally poorly defined for most products.

Storage recommendations for capsicums and chillies usually state that they should never be stored below 7^{o}C, with maximum storage life around 8-9^{o}C (Lloyd and Ryall, 1978, Kader, 2002). The results of this trial, as well as previous experiments, indicate that modern varieties are actually not very chilling sensitive. Red capsicums and red chillies in particular appear to be highly resistant to chilling damage (Ekman and Pristijono, 2010). Quality and storage life may therefore be improved simply by lowering storage temperatures from current levels to approximately 2^{o}C.

While the HWS may reduce product deterioration even in the absence of chilling damage, the benefits are more subtle. The appearance of rots and stem browning are certainly reduced using the HWS. At the same time, however, this trial has demonstrated that there are a number of practical difficulties in the commercial application of this method, despite success in the laboratory. These include;

- The large volume of water required for thorough drenching of commercial quantities of product
- The cost of heating water and difficulties with maintaining accurate temperature control
- OH&S issues relating to heat, steam and spray
- Issues relating to recirculating of used water with / without sanitisers added

Within a normal marketing chain, where storage times are relatively short, the costs and difficulties of applying a HWS are likely to exceed the benefits. The HWS is mainly likely to be cost effective if;

- Products are particularly chilling sensitive and / or have a short shelf life
- Products must be stored for an extended time at low temperature, whether as a quarantine treatment or to permit sea freight (for example)
- A heat source and suitable equipment can be obtained relatively cheaply.
- The HWS is used as a partial disinfestation treatment. That is, if fruit fly infestation levels are low and treatment is not mandated, a HWS could be applied to reduce the probability of infested fruit entering the supply chain. The results found in the previous chapters of this report suggest this reduction could be at least 50%.

The cost of heating was a subject of considerable discussion with the commercial partners. One possibility could be to recycle heat generated by on site cool rooms. In a hot climate such as Bundaberg, solar energy would also appear to have some possible merit. While such engineering issues are beyond the scope of this project, it could prove an interesting area for further investigation.

5.5 Key Points

- AustChilli in Bundaberg constructed trial equipment to test the effects of a hot water shower (HWS) on capsicums and chillies within a commercial setting.
- A total of 8 *C. annuum* varieties were tested; red and green capsicums, red and green ball chillies, birdseye chillies, red and green cayenne chillies and jalapenos.
- Fruit was stored at ambient temperatures (~25^{o}C) or at 8^{o}C before the trial. They were then exposed to the HWS for approx 60 seconds at 55^{o}C or processed without the hot water (controls). Packed samples were subjected to storage and transport protocols that replicated what might occur during normal marketing (total=6 days) or with the addition of a disinfestation treatment against Queensland fruit fly (total=16 days).
- Although a trend to improved quality was noted in the green capsicums subjected to a HWS, there were few statistically significant differences among the treatments.
- Quality attributes and acceptability of chillies were strongly affected by variety, HWS treatment and storage regime, initial temperature having less effect.
- Although quality on removal from storage differed among chilli varieties, loss of acceptability during shelf life occurred at a similar rate for all those tested.
- Extending storage time from 6 to 16 days to “disinfest” fruit reduced quality attributes and shelf life for all chillies.
- The HWS increased the proportion of chillies still graded as “marketable”, regardless of prior storage protocol, for up to 7 days at 20^{o}C.
- None of the chillies and capsicums tested during the trial developed symptoms of chilling injury.
- The costs of a HWS treatment system for capsicums and chillies may exceed benefits unless storage is to be for an extended period or products are more chilling sensitive than found during this trial.

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Appendix 1 - Mortality data from "most treatment tolerant lifestage" tests

		Capsicums					Capsicums + HWS			
Unit size		*2 fruit*	*2 fruit*	*2 fruit*	*3 fruit*	*3 fruit*	*2 fruit*	*3 fruit*	*3 fruit*	*3 fruit*
LPF (controls)		*140*	*78*	*64.5*	*344.3333*	*21.33*	*285.34*	*74.89*	*71.37*	*252.83*
		% mortality					**% mortality**			
Days @ 3C		*Rep 1*	*Rep 2*	*Rep 3*	*Rep 4*	*Rep 5*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 4*
EGGS	0	-	-	-	-	-	0	14.98	75.25	63.09
	2	98.21	31.41	0	0	34.38	90.01	66.17	73.85	71.65
	3	8.57	43.59	93.02	54.40	67.19	91.41	81.75	82.72	89.98
	4	85.36	69.87	100	79.86	100	94.74	87.092	93.46	100
	5	100	99.36	100	97.29	100	100	97.33	98.13	100
	6							99.11	99.07	100
	7	100	100	100	100	100	100	100	100	100
	9	100	100	100	100	100	100	100	100	100
	11	100	100	100	100	100	100	100	100	100
1ST INSTAR LARVAE	0	-	-	-	-	-	7.65	38.13	86.46	68.62
	2	61.07	34.62	11.63	22.85	28.13	37.97	51.04	88.32	69.68
	3	0	0	43.41	33.69	67.19	42.00	22.11	70.11	71.79
	4	87.14	18.59	53.49	25.27	0	73.36	75.07	80.38	93.14
	5	92.86	60.90	51.16	64.28	28.13	90.19	39.02	94.86	95.25
	6							87.98	100	99.08
	7	98.21	100	100	96.13	100	99.47	85.31	100	100
	9	100	100	100	100	100	100	100	100	100
	11	100	100	100	100	100	100	100	100	100
2ND INSTAR LARVAE	0	-	-	-	-	-	36.74	58.60	81.79	68.49
	2	7.14	70.51	0	30.49	62.5	80.55	68.40	92.53	88.13
	3	0	89.10	82.17	84.12	95.31	83.18	82.20	99.07	91.30
	4	96.43	71.15	87.60	89.25	98.44	99.47	84.87	98.60	91.30
	5	94.29	100	98.45	99.42	100	92.82	98.22	98.60	93.80
	6							99.55	100	99.87
	7	99.29	100	100	100	100	100	100	100	100
	9	100	100	100	100	100	100	100	100	100
	11	100	100	100	100	100	100	100	100	100
3RD INSTAR LARVAE	0	-	-	-	-	-	77.40	62.17	74.31	56.62
	2	25.71	66.03	57.36	49.95	65.63	40.77	62.17	94.86	89.19
	3	42.14	0	33.33	62.83	81.25	97.02	84.87	100	90.51
	4	83.93	92.31	82.17	97.00	100	99.12	86.65	98.60	94.59
	5	99.29	98.08	97.67	98.55	100	99.65	89.32	97.66	99.74
	6							94.21	100	100
	7	100	100	100	100	100	100	100	100	100
	9	100	100	100	100	100	100	100	100	100
	11	100	100	100	100	100	100	100	100	100

		Birdseye chillies		
	Unit size	*150g*	*150g*	*150g*
	Larvae / g (controls)	*1.24*	*1.07*	*4.51*
		% mortality		
	Days @ 3C	*Rep 1*	*Rep 2*	*Rep 3*
EGGS	0	-	-	-
	3	56.45	51.25	89.94
	4	93.55	60	95.27
	5	99.46	95	97.19
	6	100	100	98.67
	7	100	100	100
	8	100	100	100
	9	100	100	100
1ST INSTAR LARVAE	0	-	-	-
	3	0	48.13	55.47
	4	40.32	46.25	57.25
	5	70.43	11.88	88.91
	6	88.71	71.88	91.42
	7	100	100	99.70
	8	100	100	100
	9	100	100	100
2ND INSTAR LARVAE	0	-	-	-
	3	81.72	76.25	92.60
	4	95.16	57.5	97.63
	5	96.77	96.25	100
	6	95.70	100	100
	7	100	100	100
	8	100	100	100
	9	100	100	100
3RD INSTAR LARVAE	0	-	-	-
	3	86.02	98.13	96.89
	4	92.47	97.5	99.26
	5	96.77	100	99.85
	6	97.85	100	100
	7	100	100	100
	8	100	100	100
	9	100	100	100

		Cayenne chillies			Cayenne chillies + HWS		
	Unit size	*200g*	*200g*	*200g*	*200g*	*200g*	*200g*
	Larvae / g (controls)	*7.91*	*5.44*	*0.835*	*0.51*	*4.65*	*5.25*
		% mortality			% mortality		
	Days @ 3C	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*
EGGS	0	-	-	-	74.60	25.66	16.32
	2	34.45	53.49	47.90	17.95	51.94	53.13
	3	83.50	90.63	0	89.26	65.09	71.53
	4	98.10	98.99	76.05	61.90	95.38	91.53
	5	100	99.82	94.61	90.23	98.07	93.81
	7	100	100	99.40	76.56	99.25	97.52
	9	100	100	100	100	100	100
	11	100	100	100	100	100	100
1ST INSTAR LARVAE	0	-	-	-	57.02	36.51	42.59
	2	0	27.67	0	21.86	53.05	66.77
	3	0	14.15	39.52	59.95	61.33	78.86
	4	70.48	53.22	55.09	92.19	78.30	85.24
	5	85.40	67.65	89.82	82.42	87.00	92.67
	7	100	99	100	100	99.03	98.67
	9	100	100	100	100	100	100
	11	100	100	100	100	100	100
2ND INSTAR LARVAE	0	-	-	-	55.07	64.55	59.54
	2	90.33	19.85	0	77.53	82.88	82.96
	3	73.89	51.84	53.29	94.14	92.05	94.67
	4	86.28	92.37	76.65	100	91.62	95.43
	5	97.85	99.45	97.60	100	98.39	99.71
	7	100	100	100	100	100	99.90
	9	100	100	100	100	100	100
	11	100	100	100	100	100	100
3RD INSTAR LARVAE	0	-	-	-	98.05	68.52	70.01
	2	6.19	30.79	0	100	76.90	87.66
	3	44.75	59.38	10.78	98.05	81.09	96.48
	4	92.35	79.50	79.64	100	90.12	95.62
	5	91.40	95.96	97.60	100	93.23	96.57
	7	99.87	100	100	100	100	100
	9	100	100	100	100	100	100
	11	100	100	100	100	100	100

Appendix 2 - Temperatures during large scale disinfestations

	Replicate		Temperature probe readings (°C)								
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8 (AIR)**	***Average***
CAPSICUM	1	MAX	4.0	3.9	4.6	4.6	3.5	4.0	4.5	5.2	
		MIN	3.3	3.1	3.1	3.4	2.7	3.2	3.2	1.8	
		Average	3.4	3.1	3.2	3.6	2.9	3.3	3.3	3.0	*3.2*
	2	MAX	4.0	4.3	2.9	3.1	3.8	3.9	4.2	3.5	
		MIN	2.7	2.6	2.4	2.6	2.7	2.5	2.7	2.9	
		Average	3.0	3.0	2.7	2.8	3.1	2.8	3.1	3.0	*2.9*
	3	MAX	3.9	3.5	4.1	3.9	4.0	4.0	4.5	4.9	
		MIN	2.7	3.1	2.9	3.0	2.5	2.3	3.1	2.1	
		Average	3.0	3.1	3.0	3.1	3.0	3.1	3.2	3.3	*3.1*
	4 (HWS)	MAX	4.0	4.0	5.3	4.7	3.5	4.0	3.8	7.7	
		MIN	3.0	2.9	3.1	3.0	2.8	2.9	3.0	3.2	
		Average	3.1	3.1	3.3	3.1	2.9	3.1	3.1	4.1	*3.2*
CAYENNE CHILLIES	1	MAX	3.9	3.9	3.9	3.7	3.6	3.7	4.0	6.2	
		MIN	2.9	3.0	2.9	2.9	3.0	3.0	3.1	2.1	
		Average	3.0	3.1	3.0	3.0	3.1	3.1	3.1	3.3	*3.1*
	2	MAX	3.4	3.8	3.9	3.8	4.1	4.2	4.1	5.4	
		MIN	2.9	3.1	2.9	3.0	3.0	3.2	3.0	2.5	
		Average	3.0	3.2	3.0	3.1	3.1	3.3	3.1	3.3	*3.1*
	3	MAX	3.9	3.9	4.1	3.9	3.8	3.3	3.8	6.1	
		MIN	3.1	3.1	3.1	3.0	3.2	3.0	3.1	1.8	
		Average	3.2	3.2	3.2	3.1	3.2	3.0	3.1	3.3	*3.2*
	4 (HWS)	MAX	3.4	4.5	3.8	4.0	3.4	3.8	3.9	7.1	
		MIN	3.1	3.0	3.0	3.0	3.1	3.2	3.1	1.8	
		Average	3.2	3.0	3.1	3.1	3.1	3.2	3.3	3.1	*3.2*

Appendix 3 - Capsicum and chilli grading scales

Stem browning

0 - Plump, fresh, bright green

1 - Slight browning or shrivelling, no longer bright

2 - Moderate browning affecting 20 – 60% of stem and calyx

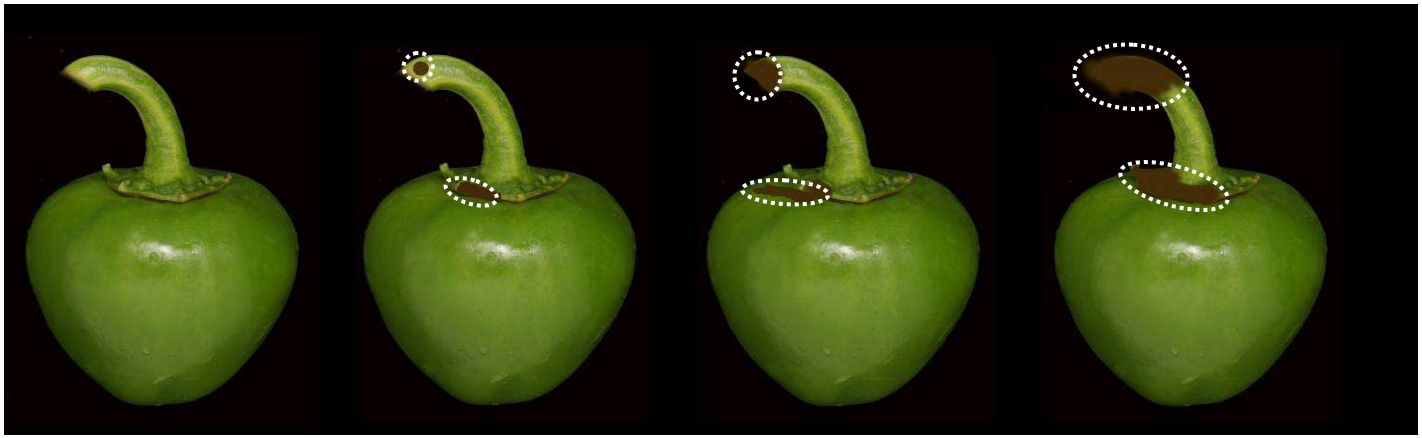
3 - Severe browning, >60% browned and shrivelled

0 **1** **2** **3**

Flesh rots

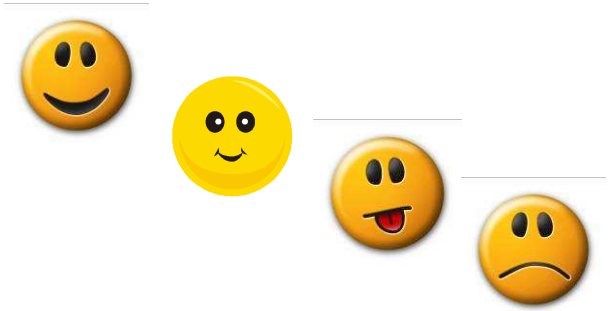
0	1	2	3
None	Slight	Moderate	Severe
Marketable	Marketable (just)	Useable (partly)	Consumer would throw out

Stem / Calyx Rots



0	1	2	3
None	Slight Small spots	Moderate, Noticeable	Severe, >50% affected Saleability greatly reduced

Overall Acceptability



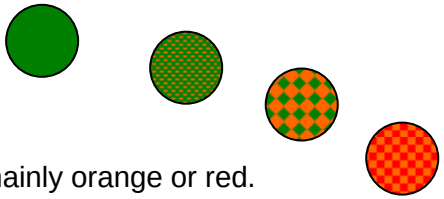
0 – Excellent, fresh

1 – Good, Marketable

2 – OK, not saleable but edible, acceptable for cooking

3 – Poor, consumer would throw away

Colour change



0 – 100% green

1 - >80% green

2 – 50 – 80% green

3 – severe degreening, mainly orange or red.